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The quality of DNA extracted from liquid or dried blood is not adversely affected by storage at 4°C for up to 24 h

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Background A consistent and stable source of DNA is an essential requirement for many Biobanks. An important pre-analytical variable is the delay between sample collection and sample processing.

Methods Fresh blood samples ($n = 80$) were collected and either processed immediately or after storage for 24 h. The samples were either stored as liquid blood at 4° C or as dried blood spots at ambient temperature on three types of paper-based storage media. The quality of the DNA extracted from the samples was measured.

Results No difference was observed between fresh and stored blood samples.

Conclusions The quality of DNA extracted from liquid or dried blood is not adversely affected by storage at 4° C for up to 24 h.

Keywords DNA, Biobank, storage, dried blood, genetic analysis

Introduction

The correlation of human genetic variation and clinical phenotype is a central element of many Biobanking projects. An essential requirement for most Biobanks is a stable source of DNA, which yields material suitable for analysis by current and future techniques.

The complete human genome sequence is available as well as tools to measure genetic variation. Although the major types of genetic variation are well known [1,2] the specific genetic changes, which will be measured in any particular study have not necessarily yet been identified. Therefore, there is a need to ensure that the collection and extraction methods used are generally applicable to the analysis of all genetic changes.

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Source of DNA

The two primary sources of human DNA that have been used in genetic analysis studies are white blood cells and cheek cells from saliva, mouthwash or swabs. Whereas the latter is non-invasive and convenient for the subject and provides DNA, which is suitable for genotyping it suffers from the limitations that there are significant levels of bacteria and viruses in the sample leading to the contamination of the sample with non-human DNA. Furthermore, the yields of DNA are variable and there are also potential long-term storage stability issues due to microbial growth.[3] For this reason only blood was tested as a sample source.

Storage of DNA

Ideally a sample would be taken from a subject and placed immediately into storage but in practice there will always be a delay between sample collection and sample processing. It is important to determine whether this delay introduces changes into the results obtained from the sample. Therefore, a first aim of this study was to assess the impact, on extracted DNA quality and quantity, of the storage of blood at 4°C for up to 24 h prior to the harvesting of white blood cells

or the spotting of blood onto a blood collection card. The procedures for blood collection and storage at t(time) = 0 and $t = 24$ have been described earlier in this volume.[4]

Both blood and extracted genetic material can be successfully stored for many years at −80 or −120°C.[5] Although this is a reliable method it may not be the most cost effective in the context of a Biobanking project as it requires expensive sample handling systems, which can operate at low temperature. An alternative is the use of room temperature storage using dried samples. Data is available to indicate that DNA can be successfully extracted from dried blood spots, which have been stored for at least 10 years.[6,7] The use of dried blood spots could introduce considerable flexibility into the operational aspects of a Biobank therefore, a second objective was to compare the suitability of DNA extracted from blood collection cards and white cells for current analytical and amplification techniques and anticipated future developments.

Methods

DNA extraction methods

Eighty EDTA blood samples were extracted by each method (40 subjects, $t = 0$ and $t = 24$).

White blood cells

The QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) was used, according to the manufacturer's instructions, to extract DNA from white blood cells harvested from 3.75 ml of blood.

For all three types of papers (Biospot, FTA and 903) 20 µl of blood was spotted onto the card and allowed to air dry at ambient temperature for 2 h.

Biospot (now marketed as FTA-Elute)

Three 3 mm diameter circles were added to a tube, washed with 500 µl sterile water and vortexed three times. Excess water was removed and the wash procedure repeated twice. Two-hundred micro liters of water was added and the sample was vortexed again before transferring to a heating block for 30 min at 100°C. The sample was then centrifuged again and the eluate containing the DNA removed.

FTA

Three 3 mm diameter circles were added to a tube and washed with 200 µl of FTA purification reagent, then incubated for 5 min at ambient temperature. The wash procedure was repeated a further three times, then 200 µl TE-1 Buffer was added and incubated for a further 5 min. This stage was repeated and the discs dried. Seventy microliters of Solution 1 was added to the discs and incubated for 5 min then 130 µl of Solution 2 added. The eluate was removed following two further rounds of vortex and incubation.

903

Three 3 mm diameter circles were added to a tube and washed with 200 µl of FTA purification reagent, then incubated for 5 min at ambient temperature. The wash procedure was repeated twice, then 200 µl TE-1 Buffer was added and incubated for a further 5 min, this stage was repeated and the discs dried. Seventy microliters of Solution 1 added to the discs and incubated for 5 min then 130 µl of Solution 2 added the eluate removed following incubation and vortexing.

DNA assessment methods

DNA yield was assessed using a real-time PCR method. The Ct (threshold cycle) of the test sample was compared against a standard curve constructed using dilutions of human genomic DNA of known concentration.

DNA length was assessed by PCR amplification with three primer sets generating fragments of 10 kb, 5 kb and 2.1 kb.

DNA purity was assessed by adding extracted DNA to a real-time PCR reaction designed to specifically amplify the mouse actin gene. The presence of inhibitors was detected by observing the difference in Ct between reactions containing no human DNA and those to which the extracted DNA had been added.

Whole Genome Amplification (WGA) was performed using the RepliG WGA kit (Qiagen GmbH, Hilden Germany) according to the manufacturer's instructions.

Sequence analysis methods

A 340 bp fragment of the BCHE gene flanking the K variant G>A at position -140 was amplified by PCR and sequenced using standard methods.

SNPs were analysed using two homogeneous fluorescent methods. In the first the NAT2*5 allele was measured using a two tube allele specific real time PCR assay. In the second the UGT2B15*2 allele was detected using an allele specific end-point PCR method.

DNA (1 µg) extracted directly from white blood cells or from successful WGA of blood spot DNA was dispatched to Affymetrix Inc, (Santa Clara) for analysis using the GeneChip Mapping 100 K set.

Results

Selection of storage materials

There are currently two DNA storage papers on the market—FTA and FTA Elute (Whatman). At the time of the project FTA elute was not available, therefore an alternative version of FTA Elute, known as BioSpot, was used. Both of these papers include chemicals designed to stabilize the DNA, for

Table 1 Yield of DNA from the three paper based methods at $t=0$ and $t=24$

Medium	Time	Average total yield (ng)	Std Dev yield (ng)	Max total yield (ng)	Min total yield (ng)
Biospot	all	121	60	302	9
	$t=0$	120	54	255	31
	$t=24$	122	67	302	9
903	all	58	41	242	14
	$t=0$	46	25	108	18
	$t=24$	70	51	242	14
FTA	all	120	46	232	32
	$t=0$	115	48	232	32
	$t=24$	123	44	218	55

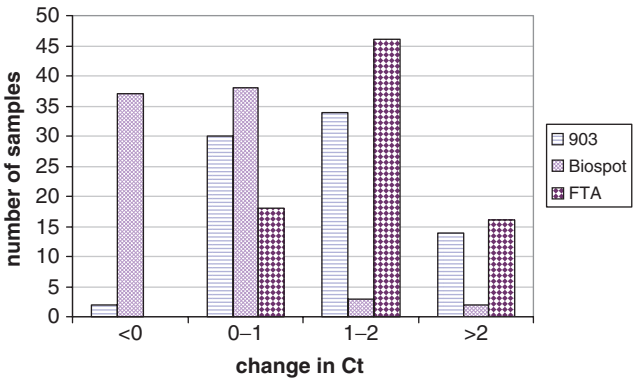

Figure 1 Distribution of samples, for each card type, showing the increases in threshold cycle (Ct) observed in the inhibitor assay

comparison 903 paper (S&S) which does not contain additives was also tested.

DNA yields

As expected good amounts of DNA were obtained from white blood cells extracted using the QIAamp kit (up to 17 µg) with no significant differences between $t=0$ and $t=24$ time points. It was observed that isolation of the white cell containing buffy coat could be a important variable in the process and failure to collect sufficient material could lead to lower than expected DNA yields. The amounts of DNA extracted from the three different papers are shown in Table 1. Although the same amount of blood (20 µl) was spotted onto each paper the areas of the spots were not uniform varying from between 42 and 62 mm^2. This means that the three punches contained between 6.5 and 9.5 µl of blood.

DNA quality

DNA quality was assessed using assays for length and purity.

No significant change of Ct was observed in the inhibition assay at either $t=0$ or $t=24$ in the QIAamp extracted DNA. The levels of inhibitor in DNA extracted from the three papers are shown in Figure 1. Both FTA and 903 DNA resulted in a Ct retardation of at least 1 Ct in the majority of samples whereas this level of inhibition was seen in only 5/80 Biospot extracted DNA samples. A Ct retardation of 1 is indicative of a reduction in PCR efficiency of ~5%.

The length of DNA extracted from the white cells was around 20 kb as estimated by agarose gel electrophoresis. No difference was observed between $t=0$ and $t=24$.

The 2.1 kb amplicon was observed in 80/80 of the FTA extracts and 71/80 of the Biospot extracts although neither FTA nor Biospot yielded 10 kb or 5 kb fragments. The largest fragment that could be amplified from 903 extracted DNA was 5 kb for 31 samples out of 80 tested.

Whole genome amplification

All of the white cell extracted DNA and all of the FTA DNA was successfully used for WGA whereas neither the 903 paper nor the Biospot paper yielded DNA that was suitable. No difference was observed between $t=0$ and $t=24$.

Sequence variation analysis

The amplification of the region to be sequenced resulted in a PCR fragment of 340 bp. The resulting sequencing traces were between 310–320 bases long. Nine samples did not give a clear sequence at the 5′ end for the first 30–40 bases. Eight of these samples were extracted from FTA cards and one from Biospot. No significant difference was observed between $t=0$ and $t=24$. All DNA samples were successfully genotyped using the homogeneous fluorescent methods. The amplicon size for the NAT2*5 assay was 90 bp and 166 bp for the UGT test.

Sequence analysis using the GeneChip Mapping 100 K set required 1 ug of DNA/sample. The yields from the cards were too low to allow direct analysis so whole genome amplified samples were used. As only FTA paper was compatible with WGA it was not possible to test Biospot and 903 DNA in this system. The Mapping 100 K assay performed very well on all samples tested, genomic DNA as well as WGA. No significant variation was observed between $t=0$ and $t=24$ h samples but as the sample numbers were low a low level difference cannot be ruled out.

Discussion

There was no observable decrease in yield, fragment length and purity of DNA extracted from samples at $t=0$ and $t=24$. The ability of the samples to be

genotyped by homozygous fluorescent and heterozygous fluorescent methods was not impaired by storing the blood for 24 h at 4°C. The ability to sequence was not inhibited or unduly affected by the aging of the blood at 4°C. This was true regardless of method of extraction of DNA or method of blood collection (white cell buffy coat or paper storage media). In this regard a 24 h gap between sample collection and processing is acceptable.

All three of the card systems provided DNA suitable for simple genotyping but suffered in comparison to white cell DNA for more exacting applications. The data on length and purity suggest a cause for this. The Biospot (FTA Elute equivalent) cards generated DNA that was relatively free of inhibitors but was fragmented and could not generate long DNA fragments. In contrast the DNA from the FTA and 903 papers was longer but contained inhibitors that could interfere with applications such as DNA sequencing. The most promising of the papers was FTA Elute and its performance for Biobanking could be further improved by development of a gentler extraction procedure, that would minimize fractionation. The manufacturer's website claims that DNA extracted from FTA elute is compatible with WGA procedures (http://www.whatman.com/repository/documents/s7/51690%20(S9036-805).pdf)

In summary it is possible to use paper based systems for Biobanking applications but the amount and quality of DNA that they yield is, not surprisingly, inferior to DNA extracted from liquid blood. Some of the limitations of yield and quality can likely be overcome by whole genome amplification but whilst this would allow SNP genotyping it would not be suitable for copy number determination due to differential amplification efficiencies. As the establishment of a Biobank is typically a substantial undertaking it is likely that the extra effort and inconvenience involved in the collection and shipping of liquid blood is justified in terms of a more robust resource for future applications.

Conflict of interest: None declared.

References

[1] Redon R, Ishikawa S, Fitch KR *et al*. Global variation in copy number in the human genome. *Nature* 2006; **444:**444–54.

[2] Database of Single Nucleotide Polymorphisms (dbSNP). Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID:126. Available at: http://www.ncbi.nlm.nih.gov/SNP/ (Accessed February, 2007).

[3] Feigelson HS, Rodriguez C, Andrea S *et al*. Determinants of DNA yield and quality from buccal cell samples collected with mouthwash. *Cancer Epidemiol Biomar Prev* 2001;**10:**1005–8.

[4] Peakman TC, Elliott P. The UK Biobank sample handling and storage validation studies. *Int J Epidemiol* 2008; **37(Suppl 1):**i2–i6.

[5] Visvikis S, Schlenck A, Maurice M. DNA extraction and stability for epidemiological studies. *Clin Chem Lab Med* 1998;**36:**551–55.

[6] Freeman N, Smith C, Curtis L, Huckett J, Mill J, Craig IW. DNA from buccal swabs recruited by mail: evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping B. *Behav Genet* 2003;**33:**67–72.

[7] Chaisomchit S, Wichajarn R, Janejai N, Chareonsiriwatana W. Stability of genomic DNA in dried blood spots stored on filter paper. *Southeast Asian J Trop Med Public Health* 2005;**36:**270–73.